PROSPECTS
Journal of Cellular Biochemistry 117:29–38 (2016)

Journal of Cellular Biochemistry

Methyl-CpG-Binding Protein (MBD) Family: Epigenomic Read-Outs Functions and Roles in Tumorigenesis and Psychiatric Diseases

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ABSTRACT

Epigenetics is the study of the heritable changes on gene expression that are responsible for the regulation of development and that have an impact on several diseases. However, it is of equal importance to understand how epigenetic machinery works. DNA methylation is the most studied epigenetic mark and is generally associated with the regulation of gene expression through the repression of promoter activity and by affecting genome stability. Therefore, the ability of the cell to interpret correct methylation marks and/or the correct interpretation of methylation plays a role in many diseases. The major family of proteins that bind methylated DNA is the methyl-CpG binding domain proteins, or the MBDs. Here, we discuss the structure that makes these proteins a family, the main functions and interactions of all protein family members and their role in human disease such as psychiatric disorders and cancer. J. Cell. Biochem. 117: 29–38, 2016.

KEY WORDS: METHYL-CpG BINDING PROTEIN; EPIGENETICS; DNA METHYLATION; CANCER; AUTISM; SCHIZOPHRENIA

Epigenetics is a term coined by the developmental biologist Waddington in the 40′s to promote the importance of genetics as an underlying factor in biology. At the time, this new field focused on studying genetics and heredity during embryology [Waddington, 1942]. Currently, epigenetics is defined as the study of heritable changes in short term in gene expression that are not explained by DNA mutations. Epigenetic marks and mechanisms include DNA and histone modifications, chromatin remodeling and noncoding RNA regulation. The most studied epigenetic mark is DNA methylation. During development, the genome undergoes dynamic and complex changes in DNA methylation patterns, occurring during early embryonic life; for example, genomic imprinting during embryo development and tissue-specific epigenetic profiles for specific gene expression, as well as during adult life, such as the formation and maintenance of memory [Lopez-Serra and Esteller, 2008; Nagy and Turecki, 2012]. DNA methylation is the result of a cytosine residue that is modified by a methyl group at the 5′ position of the pyrimidine ring (5 mC) [Bird and Wolffe, 1999]. These dynamic patterns of DNA methylation are read by specific proteins that play a role in the interpretation of these marks. These proteins are called methyl-CpG binding domain proteins and are grouped in three structural families: (1) the SET and RING finger–associated domain (SRA) family that binds to hemimethylated DNA with a recognition step involving the flipping of methylcytosine out of the DNA helix [Arita et al., 2008]; (2) the zinc finger family with a Kaiso binding site that binds to methylated and non-methylated DNA [Daniel et al., 2002]; and (3) the MBD family with a conserved methyl-CpG binding domain (MBD) homology and with the first member being identified in 1989, [Meehan et al., 1989]. DNA methylation is generally associated with regulation of gene expression through repression of promoter activity and with genome stability [Buck-Koehntop and Defossez, 2013]. DNA methylation abnormalities have been described in many types of cancer [Gigek et al., 2012; Lopez-Serra and Esteller, 2008] and in psychiatric disorders [Gavin et al., 2013; Nagy et al., 2015]. Moreover, the ability of the cell to interpret the correct methylation marks and/or the correct interpretation of methylation plays a role in many diseases. Thus, it is of equal importance to understand how the epigenetic machinery acts in both normal and disease conditions. Therefore, our aim is to review and gather information about the MBD family members to provide the

Grant sponsor: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Grant sponsor: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Manuscript Received: 17 July 2015; Manuscript Accepted: 20 July 2015

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 23 July 2015

DOI 10.1002/jcb.25281 • © 2015 Wiley Periodicals, Inc.

most recently reported data concerning their functions and roles in epigenetic mechanisms and how the imbalance of these proteins can affect tumorigenesis and neurological disorders.

MBD FAMILY

The identification of the first MBD family member was published in 1992 [Lewis et al., 1992]. The authors described a single methyl CpG binding protein. At that point, the only known protein, called MeCP1, required a group of at least 12 methylated sites for efficient [Meehan et al., 1989]. The novel protein was named as a family member of the MeCP family, MeCP2. However, later, it was established that MeCP1 represses transcription by recruiting histone deacetylases and corepressor proteins and that the MeCP consensus binding sequence is different than that for methyl-CpG binding proteins [Nan et al., 1998; Ng et al., 1999].

The current classification is that MBD proteins can bind selectively to methyl-CpG pairs, although some have differential affinity for methylated DNA. Additional members of this family have been identified based on conserved amino acid sequences that are homologous to the MBD domain of MeCP2: MBD1-6 [Hendrich and Bird, 1998; Laget et al., 2010] (Fig. 1). These proteins have a conserved MBD sequence from human to mouse, and the solved structure of the MBD domain bound to DNA showed that the protein binds as a monomer and possesses a wedge shaped form made of four β-sheets superimposed on an α-helix and hairpin loop, known to contact the DNA backbone, while conserved residues within the β-sheets recognize the methyl group [Wade and Wolffe, 2001]. Thus, MBD proteins can bind methylated CpG dinucleotides regardless of the sequence context [Hendrich and Bird, 1998]. By definition, the methyl binding domain consists of about 70 residues and is the minimal region required for binding to methylated DNA by a methyl-CpG-binding protein which binds specifically to methylated DNA. The MBD can recognize a single symmetrically methylated CpG either as naked DNA or within chromatin [Marchler-Bauer et al., 2015].

The family founder, MeCP2, has the ability to bind to a single symmetrically methylated CpG both in pure DNA and within chromatin [Bird and Wolffe, 1999]; and to mCpGs that are flanked by A/T residues [Klose et al., 2005]. The *MeCP2* gene, located on Xq28, is composed of four exons and gives rise to two alternatively spliced variants, MeCP2α and MeCP2β, depending on the presence or absence of exon 2 [Lopez-Serra and Esteller, 2008]. *MeCP2* is found to be expressed ubiquitously in various tissues but is most abundant in brain, and gene mutations lead to Rett syndrome, a neurological disorder. *Mecp2*-null mice studies clarified an essential role of this protein in striatum, hypothalamus and cerebellum, as its absence was associated with reduced brain size and body weight, abnormalities in locomotor and gait activities and breathing deficits [Guy et al., 2001; Zhao et al., 2013]. A non-neuronal role for MeCP2 has also emerged in myofibroblast differentiation [Mann et al., 2010], lung development [Joss-Moore et al., 2011] and mesenchymal stem cells proliferation [Squillaro et al., 2010]. Metabolic disorders were also highlighted as a consequence of *Mecp2* deletion [Pitcher et al., 2013; Zhao et al., 2013].

The DNA binding activity of MeCP2 was explored in the brain through a genome-wide approach, revealing a profile that tracks DNA methylation density throughout the genome [Skene et al., 2010]. The transcriptional repression activity of MeCP2 depends on DNA methylation, and after a demethylation drug treatment, gene reactivation overlaps with a loss of MeCP2 [El-Osta et al., 2002]. However, MeCP2 absence generated no significant alterations or just a small number of misexpressed genes in the brain, thereby supporting the idea of an interaction between MeCP2 and transcriptional activators. Yet, a list of deficient proteins is found in different studies with MeCP2-deficient mouse models [Guy et al., 2011].

The group that first isolated this protein considered two alternative views for MeCP2 function in the brain: as a regulator of development or as a factor that helps maintain neuronal/glial function [Guy et al., 2011]. MeCP2 distribution in the genome, however, has consequences for global chromatin structure, as data also showed that MeCP2 can associate with nucleosomes, affecting the acetylation level of entire chromatin complement [Guy et al., 2011]. Therefore, the complex molecular function and MeCP2 involvement in brain function raises a large number of questions and remains to be intensively studied.

MBD1, initially called PCM1, also binds specifically to methylated CpG and localizes to the nucleus to enhance heterochromatin formation [Hameed et al., 2014]. The *MBD1* gene is located on chromosome 18q21 and is very close to *MBD2* [Hendrich et al., 1999]. Uniquely among MBD proteins, MBD1 has two to three cysteine-rich CXXC domains (Fig. 1), originally found in DNA

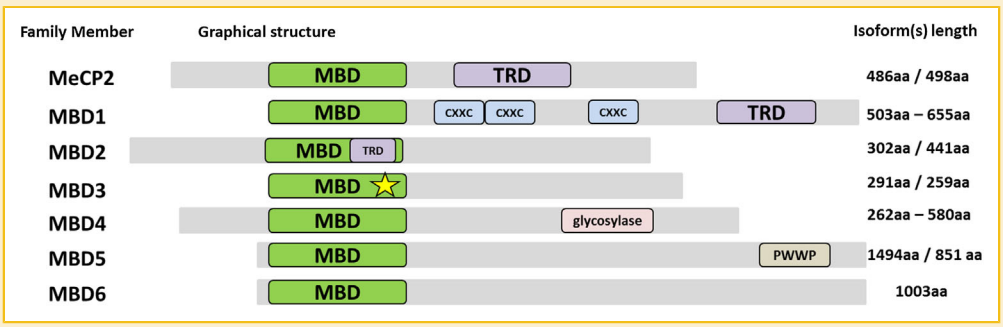

Fig. 1. A scheme of the seven components of the methyl binding domain (MBD) protein family and protein interaction domains. The yellow star represents the two distinct amino acid residues in the MBD domain of MBD3 (MBD, methyl-CpG-binding domain; TRD, transcriptional repression domain; CXXC, Cys-x-x-Cys domain; PWWP, proline-tryptophan-tryptophan-proline domain.

methyltranferase and implicated in mediating binding to unmethylated CpG. The thirteen MBD1 isoforms described so far are alternatively spliced in the region of the CXXC domain. Isoforms with three domains may repress gene expression from both methylated and unmethylated promoters; whereas isoforms with two motifs appear to inhibit transcription only in methylated promoters. MBD1 also acts as a transcriptional regulator depending on the density of methyl CpG [Fujita et al., 2000]. One variant with the CXXC-3 domain and a deletion of the entire MBD domain was reported having a strong affinity toward unmethylated CpG islands as well as a concomitant loss of binding to methylated CpG islands [Baubec et al., 2013]. Therefore, MBD1 makes use of two distinct DNA binding domains to target CpG; the MBD recognizes methylated CpG and the CXXC-3 domain binds to nonmethylated CpG [Jorgensen et al., 2004].

Through all of these structural characteristics, MBD1 is reported as crucial for gene silencing in essential cell functions, as cell division and differentiation. *Mbd1*-null mice present impaired adult hippocampal neurogenesis and increased genomic instability in neural stem cells, although healthy and fertile [Zhao et al., 2003]. Similar to MeCP2, MBD1 is an abundant chromosomal protein, detected at the methylated allele of imprinted genes [Hendrich and Tweedie, 2003]; moreover both proteins contain a powerful transcriptional repression domain (TRD) (Fig. 1) [Jones et al., 1998]. The TRD recognizes binding partners and appears to communicate with histone deacetylase (HDAC). Due to recruitment of these complexes, MBD1 overexpression is often linked to carcinogenesis, resulting in the silencing of tumor suppressor genes [Hameed et al., 2014].

Likewise, MBD2 also contains the TRD domain in its structure [Hendrich and Bird, 1998] and the removal of this domain is implicated in the disruption of the MBD domain, leading to loss of function [Boeke et al., 2000] (Fig. 1). The gene located on 18q21 [Hendrich et al., 1999] has a large CpG island, although the control of MBD2 expression was not associated exclusively to promoter DNA methylation in gastric tissue [Pontes et al., 2014]. Two isoforms, the longer MDB2a and MBD2c, differ in the C-terminal region and were recently shown to have a role in the self-renewing state of stem cells, both enriched at promoter of two master pluripotency regulators, *OCT4* and *NANOG*. MBD2c overexpression enhances the reprogramming of fibroblasts to pluripotency, whereas MBD2a preferentially interacts with the repressive NuRD chromatin remodeling factors, promoting stem cell differentiation [Lu et al., 2014]. The NuRD is a nucleosome remodeling and HDAC complex with a well-established role in development and is recruited by MBD2, leading to gene silencing through DNA methylation [Lai and Wade, 2011].

MBD2 also has the ability to bind to methylated CpG sequences in vitro [Hendrich and Bird, 1998] and in vivo [Chatagnon et al., 2011]. MBD2 shows a strong preference for highly methylated regions, overlapping promoters and transcriptional start sites [Chatagnon et al., 2011; Menafra et al., 2014], but is also irrespective of their location, such as the gene body, and being able to dramatically compact a widely expanded euchromatin [Gunther et al., 2013]. Conversely, only MBD2a was shown to associate with unmethylated DNA sequences [Fujita et al., 2003]. Upon MBD2 depletion, genes bound by this protein were found to be activated, therefore supporting the repressive role of MBD2 binding to methylated CpG [Gunther et al., 2013]. Although Mbd2-knockout mice are viable, they display a weak phenotype, including reduced size and behavioral defects [Hendrich et al., 2001]. Additionally, as a very active protein in the epigenetic machinery, MBD2 has an important role in tumorigenesis, participating in the silencing of tumor suppressor genes [Berger and Bird, 2005].

Overall, MBDs have a sequence similarity limited to the MBD domain itself, with the exception being MBD2 and MBD3 (with approximately 70% overall amino acid identity) [Berger and Bird, 2005]. The key difference is that MBD3 fails to bind methylated CpG both in vitro and in vivo [Baubec et al., 2013; Hendrich and Bird, 1998], most likely due to two distinct amino acid residues in the MBD domain (Fig. 1); these substitutions, however, appear to be evolutionary necessary and sufficient for physical interaction of MBD3 with the NuRD complex. Also in the NuRD complex context, MBD2 and MBD3 can also form a heterodimer with a single CpG interaction domain provided by MBD2 and two potential domains for interaction with other proteins by MBD3 [Saito and Ishikawa, 2002]. NuRD complexes containing either MBD2 or MBD3, or both, may be targeted to regions of the genome with distinct epigenetic marks. Further, MBD3 was demonstrated capable to bind to 5-hydroxymethylation, an intermediate stage in cytosine demethylation, which also functions as a regulatory mark for gene expression [Yildirim et al., 2011].

The *MBD3* gene is located on 19p13 [Hendrich et al., 1999], codes for the smallest member of the family [Berger and Bird, 2005], with two isoforms [Jung et al., 2003]. MBD3 is crucial to normal mammalian development, as *Mbd3* knockout in mice was embryonically lethal [Hendrich et al., 2001], suggesting that MBD3 is essential to early embryonic development and also at late embryonic stages. A spatial and temporal pattern of *MBD3* expression was described in the developing brain, with the shorter isoform being the most prevalent in these embryonic neural cells [Jung et al., 2003], as well as in proliferating HeLa cells [Ng et al., 1999]. However, the two isoforms of MBD3 appear to co-exist in cells and tissues, and it is not clear whether the two forms function independently of each other.

MBD3 interacts directly with rRNA genes for normal cell function by binding to their unmethylated promoters via transcription factor UBF. This essential role in rRNA expression regulation is consistent with embryo-lethality, as MBD3 knockdown in HeLa cells shows increased promoter methylation to which PolI is unable to bind [Brown and Szyf, 2007]. MBD3 was also unexpectedly shown to interact with active fractions of the genome, locating in active promoters associated with histone marks to open chromatin and with genomic regions bearing enhancers. Hence, the current models for the NuRD complex as a static corepressor are inadequate in the face of emerging genomic data about MBD components [Shimbo et al., 2013]. Additionally, MBD3 overexpression resulted in higher NuRD levels and facilitated cell reprogramming through gene activation during the process, and loss of MBD3 led to reduced reprogramming efficiency [dos et al., 2014].

MBD4 has a slightly different function than other MBD components. The deamination of methylcytosine to thymine yields mutagenic mispairs and is a major source of C → T point mutations at CpG sites. MBD4 has a glycosylase domain (Fig. 1) and is the most likely candidate to remove the T or U when it is present in a mismatched base pair with G without cleaving the DNA strand. Its

MBD domain can bind symmetrically to methylated CpG sites and has a higher affinity for 5mCpG/TpG mismatches [Hendrich and Tweedie, 2003]. The *MBD4* gene maps to 3q21, and *Mbd4* inactivation in mice causes increased CàT transitions and promotes tumor formation, thereby indicating that MBD4 suppresses CpG mutability and tumorigenesis in vivo [Millar et al., 2002]. Therefore, the ability of MBD4 to perform the identification and excision of deaminated methyl cytosine is important for the maintenance of genetic and epigenetic integrity.

Although not fully explored, MBD4 is described as a transcriptional repressor, and targets the genes *MLH1*,*p16*, *p21*, *MSH4*, and *cMYC*. MBD4 is involved in cell death signaling by interacting with agents to DNA damage response and also plays a function during oxidative stress, along with DNA methyltransferase 1 (DNMT1), both recruited to DNA damage sites [Laget et al., 2014].

MBD5 and MBD6 were the last two family members to be characterized and functional studies revealed that they cannot bind to methylated CpG [Laget et al., 2010], as MBD3. The *MBD5* gene, on 2q23, is disrupted in patients with neurobehavioral abnormalities and associated with a microdeletion syndrome [Talkowski et al., 2011; Talkowski et al., 2012]. Mice with significantly reduced *Mbd5* mRNA resulted in lower body weight and length, impaired growth pathway signaling and died perinatally [Du et al., 2012]. Although MBD5 cannot bind directly to methylated DNA, it was shown to associate with heterochromatin; therefore, MBD5 might bind to DNA in a complex as it interacts with MEF2C, which plays a crucial role in development, neurogenesis and neuronal gene regulation [Li et al., 2008]. *MEF2C* haploinsufficiency results in severe mental retardation, with a phenotype similar to MBD5 deficiency [Nowakowska et al., 2010].

MBD5 codes for two isoforms, isoform 1 containing a PWWP domain, not present in isoform 2 (Fig. 1). This domain is required for recruitment to the methylated pericentric heterochromatin, as confirmed by MBD5 isoform 1 localization to chromocenters, whereas isoform 2 is not. Although MBD5 isoform 1 is expressed in all tissues, with lowest mRNA levels in early embryos and highest in the brain and testis, the isoform 2 is relatively homogeneous in all tissues and is very high in oocytes. Therefore, MBD5 might contribute to the epigenetic machinery of neurons or to the global reorganization of chromatin during spermatogenesis [Laget et al., 2010].

MBD5 and MBD6 also share a specific feature of their MBD domain, necessary for the interaction with proteins: the Polycomb repressive complex (PR-DUB), first identified in *Drosophila,* that plays a role in catalyzing the deubiquitination of H2A at lysine (K) 119. This interaction, however, is not required for the MBD6 recruitment to sites of DNA damage [Baymaz et al., 2014]. The role of MBD6 at DNA damage sites remains unknown, and further investigation is required.

The *MBD6* gene, on 12q13, is expressed ubiquitously, with the highest in testis and the lowest in ovary. As a binding protein, MBD6 has a heterogenous subnuclear localization, and the MBD domain is necessary for its recruitment to pericentric heterochromatin, although it does not bind to methylated *CpG* in vitro [Laget et al., 2010] (Fig. 1). Nevertheless, MBD6 was recently attributed a major role as a crucial master regulator of MBD family member, hypothesized to interact with MBD2-4 and MBD6 itself. Moreover, knockout cells for *MBD6* had attenuated cell proliferation and were eventually induced to cell death, therefore MBD6 plays a role in cell differentiation and proliferation as a direct target of Oct4 [Jung et al., 2013].

A summary of the main characteristics of the MBD family is shown in Table I. It is important to notice that all members possess the conserved MBD domain as a unifying feature (Fig. 1). However, not every MBD binds to methyl CpG, as illustrated by MBD3, MBD5, and MBD6.

MBD IN CANCER

As fundamental read-outs of epigenetic marks, a growing number of studies is trying to elucidate the role of the MBDs in cancer. Epigenetic marks are commonly altered in cancer and, more specifically, aberrant DNA methylation. In general, when these regions become methylated, they are associated with gene silencing. Therefore, abnormal DNA methylation is an alternative method to mutation, both resulting in changed gene function, which is profoundly altered in cancer cells [Gigek et al., 2012; Lopez-Serra and Esteller, 2008]. Moreover, differential histone modifications have also been described to work together with DNA methylation in cancer [Gigek et al., 2012], and MBDs have been demonstrated to crosstalk with these two important epigenetic mechanisms [Berger and Bird, 2005].

In this section, we focused on the recent and overall functions of the MBDs and how these proteins interact with epigenomic marks in specific genes and in certain type of tumors. The knowledge of targets of MeCP2 and MBD1-4 is currently narrow, and has been previously reviewed elsewhere [Lopez-Serra and Esteller, 2008].

MeCP2 is not expressed during proliferation or in vitro differentiation of embryonic stem cells, but it becomes important at the organ differentiation stage. A significant upregulation of *MeCP2* and *MBD2* mRNA was shown during prenatal development of the human mammary gland [Billard et al., 2002]. In breast cancer, lower *MeCP2* mRNA expression was found in non-neoplastic tissue when compared to cancer tissue; moreover, a positive association between levels of *MeCP2* mRNA and estrogen receptor was also found [Muller et al., 2003]. In contrast, another study reported reduced expression of isoform MeCP2β in breast cancer when compared to the adjacent tissue, losing a crucial mechanism of suppression that leads to the overexpression of ADAM-12, a multifunctional protein involved in the cancer-metastasis cascade [Ray et al., 2012]. More investigations are needed in order to elucidate the exact role of MeCP2 targets and confirm isoforms in this type of cancer.

Besides breast cancer, MeCP2 was also shown to be critical for other tumorigenesis. It is possible that MeCP2 interacts with transcription factors and oncoproteins, thereby contributing to carcinogenesis in cancer cells. In prostate cancer, MeCP2 contributes by transactivating stress survival genes, directly binding to promoter sites, and enhancing their activation[Leoh et al., 2012]. In neuroblastoma, ChIP (Chromatin Immunoprecipitation) experiments revealed MeCP2 to interact, by association with another oncoprotein MYCN, with the promoter regions of important genes for the development of this cancer type [Murphy et al., 2011]. In a type of lymphoma, the silencing of a methylated proapoptotic protein of the Bcl2-family gene (*BIM*) occurs through recruitment of MeCP2 and the SIN3a/HDAC1/2 corepressor complex [Piazza et al., 2013]. Additionally, MeCP2 can also recruit a H3K9 methyltransferase, thus strengthening the repressive state, a mechanism that appears to be involved in pancreatic adenocarcinoma [Dandrea et al., 2009].

TABLE I. MBD Family Members and Their Localization, Function, and Main Interactions

Protein	Genomic Location	Isoforms (RefSeq)	Main binding	Main function	Key interactions	Knockout model
MeCP2	Xq28	2	Binds to a single mCpG	Important to neurodevelopment	Nucleosomes, deacetylation complex (through TRD domain)	Reduced brain and body weight, and locomotor, gait, and breathing deficits. Metabolic disease.
MBD1	18q21	13	Methylated and unmethylated CpG (CXXC domain)	Cell division and differentiation	HDAC (through TRD domain)	Impaired adult hippocampal neurogenesis, increased genomic instability, fertile.
MBD2	18q21	2	Binds to methylated CpG	Stem cell pluripotency, gene expression repression	NuRD complex	Reduced size and behavioral defect.
MBD3	19q13	2	Binds to 5-hydroxi-mCpG	Normal development, cell reprograming	NuRD complex	Embryonic lethal.
MBD4	3q21	5	Binds to 5mCpG/TpG mismatch	Identification and excision of deaminated CpG	DNA methyltransferase 1	Enhanced tumor formation.
MBD5	2q23	2	Binds to chromocenters, and methylated pericentric heterochromatin	Development, neurogenesis and neuronal gene regulation	MEF2C PR-DUB domain	Reduced body weight, and length, died shortly after birth
MBD6	12q13	5	Binds to chromatin	Cell differentiation and proliferation	MBD2, MBD3, MBD4, MBD6	–

Therefore, MeCP2 regulation and normal expression and function are crucial to regulate gene expression, mostly by repressive effect of transcriptional activity and recruitment of epigenetic machinery.

The silencing of *MeCP2* and *MBD1* in prostate cancer cells produces cellular phenotypes that are markedly different: MeCP2 absence reduced cell growth and apoptosis, while MBD1 silenced cells showed more invasive and migratory potential via a discrete set of genes with increased mRNA expression [Yaqinuddin et al., 2008]. Moreover, repression of methylated genes was associated with MBD1 expression, and hypothesized to work along with DNMTs and HDACs resulting in tumor initiation and progression [Patra et al., 2003]. Then, we conclude MBD1 couples DNA methylation to transcriptional repression by binding to methylated CpG islands and by recruiting methyltransferase [Lopez-Serra and Esteller, 2008]. Nevertheless, the overexpression of *MBD1* mRNA and protein correlated with lymph node metastasis in pancreatic carcinoma [Di et al., 2006]. In pancreatic cancer cell lines, *MBD1* knockdown revealed upregulation of proteins associated with migration and invasion, and downregulation of proteins usually overexpressed in cancer, heat shock proteins, transcriptional regulators and angiogenesis factors [Liu et al., 2008]. MBD1 was validated as a potent oncogene, promoting cell invasion and metastasis through its interaction with key genomic stability and cell-cycle progression genes [Xu et al., 2013]. These findings provide insight into mechanisms of MBD1 and protein associations, a promising role to molecular targeting in pancreatic cancer therapy.

In colon and lung cancer, loss of heterozygosity has been reported on 18q21, which is the location of the *MBD1* and *MBD2* locus. This loss of function could affect the normal regulation of gene expression by lacking the complete suppression of genes that are normally silenced [Bader et al., 2003]. In colon cancer cell lines and tissue, both *MBD1* and *MBD2* showed reduced gene expression when compared to normal colon; however only *MBD2* promoter was hypermethylated and correlated to 18q21 copy number loss [Derks et al., 2009]. This controversial role of MBD1 depends on the tissue and type alteration. Additional studies are necessary to elucidate MBD1 pathways in cancer and specific tissue pathways.

The exact mechanism of tumorigenesis progression or suppression by MBD2 still remains unclear and appears to be tissue-specific. Reduced *MBD2* expression was reported in gastric cancer when compared to normal gastric mucosa [Pontes et al., 2014]. In colon cancer, MBD2 has been shown to bind to the aberrantly methylated promoter region of *p14* and *p16*, tumor suppressor genes, and to cooperate with HDAC to promote gene silencing. Besides, treatment with a demethylation agent led to the dissociation of MBD2 from their promoter and to the re-expression of these genes [Magdinier and Wolffe, 2001].

Similarly to MBD1, MBD2 silences tumor suppressor genes, frequently aberrantly hypermethylated in cancer tissues and cell lines. In breast cancer cells, MBD2 is required for this selective silencing, and when *MBD2* was knockdown an inhibition of cancer cell growth was reported, supporting a role in cancer progression [Mian et al., 2011]. In HeLa cells, MBD2 is the most abundant among the MBD transcripts [Billard et al., 2002] and is involved in the transcriptional repression of the catalytic subunit of telomerase (hTERT), as its depletion led to an upregulation of hTERT

transcription [Chatagnon et al., 2009]. In prostate cancer, *MeCP2* and *MBD2* mRNA are expressed, however no MBD2 protein is detected in cancer samples, indicating involvement of epigenetic mechanism in at the translational level regulation [Patra et al., 2003]. Accordingly, MBD2 might have an impact the cancer initiation.

Likewise, MBD3 associated with the NuRD complex has been implicated in cancer biology [Lai and Wade, 2011]. The MBD domain of MBD3 may function as a protein-protein interaction domain, as it binds to the oncoprotein JUN to recruit NuRD. Moreover, in the intestinal crypts, the inactivation of *Mbd3* expression led to higher proliferation rate and, therefore, increased susceptibility to tumor development [Aguilera et al., 2011]. In leukemia, NuRD activity is exclusively MBD3 dependent and inhibits histone deacetylation by interacting with the *Evil* gene, thus promoting gene transcription [Spensberger et al., 2008]. Moreover, MBD3 interaction with a proto-oncoprotein recruits the NuRD complex to silence *p21* by DNA methylation of its promoter in leukemia cancer cells. *p21* expression is often repressed in several cancer tissues and its promoter is frequently hypermethylated in leukemia, highlighting how *p21* is regulated in a cancer-specific manner [Choi et al., 2013]. In lung cancer, the expression levels of *p21* and *ERBB2* are regulated by MBD3-NuRD complex [Noh et al., 2005]. In gastric cancer, a strong correlation between *MBD3* and *MBD2* mRNA levels was demonstrated, requiring further investigations of their participation in the NuRD complex [Pontes et al., 2014]. Although MBD3 lacks the ability to bind to methylated CpG, it is a key component of the NuRD complex, acting as a repressor or promoting gene expression in several types of cancers [Buck-Koehntop and Defossez, 2013].

Some *MBD4* polymorphisms have been associated with higher risk of esophageal [Yin et al., 2014], cervical [Xiong et al., 2012], and colorectal cancer [Song et al., 2009], possibly somehow interfering with protein activity. MBD4 is implicated in the maintenance of chromosomal stability, and when *MBD4* is mutated, it predisposes the cell to structural chromosomal rearrangements [Abdel-Rahman et al., 2008]; however, a truncated MBD4 was reported to have a more widespread effect on genomic stability in colon cancer [Bader et al., 2007]. Moreover, mutated *Mbd4* mice show reduced mRNA expression and fail to repair CpG → TpG mutations in the *Apc* gene, an alteration commonly found in intestinal tumorigenesis, consequently accelerating tumor formation [Millar et al., 2002]. *MBD4* expression is regulated by promoter hypermethylation in colorectal and ovarian cancers, and an association between loss of mRNA expression and aberrant promoter methylation was reported in colorectal cancer in patients [Howard et al., 2009].

As MBD5 and MBD6 were more recently described, their role in reading DNA methylation marks is not well understood. There have not been any reports detailing their role or status during tumorigenesis.

Regardless of the information compiled above, no clear causal implications between MBD and any specific type of cancer can be highlighted. However, these data suggest that MBD are critical factors in tumorigenesis, interacting with key tumor suppressor and oncogenes in several types of cancers, and recruiting other epigenetic machinery enzymes to consolidate gene expression patterns. Moreover, the altered expression of a specific MBD itself can cause changes in the read-out of the DNA and trigger dysregulation of transcriptional activity, depending on the tumor type.

MBD IN PSYCHIATRIC DISEASES

Epigenetic regulation has been widely described in neurodevelopment [Lv et al., 2013] and in several processes in the brain, such as learning, memory and response to environmental stimuli [Nagy and Turecki, 2012]; hence, abnormalities in the epigenetic machinery might have a high impact on the pathogenesis of psychiatric diseases [Nagy et al., 2014; Nagy and Turecki, 2012; Talkowski et al., 2011; Talkowski et al., 2012]. Despite increased investigations into the role of MBDs in the past decade, few studies have been conducted to look specifically at psychiatric diseases. Therefore, we reviewed the implications of MBDs in schizophrenia, depression and autism spectrum disorder (ASD).

The genetic association between MBDs gene variants and psychiatric disorders has been investigated [Coutinho et al., 2007; Piton et al., 2011; Wong et al., 2014; Xie et al., 2014]. Using a cohort of trios, the rs1145317 polymorphism (*MBD2*) was found to be significantly overtransmitted from parents to schizophrenic offspring. Moreover, a haplotype including this SNP was associated with schizophrenia, suggesting that *MBD2* might be a susceptibility gene for schizophrenia [Xie et al., 2014].

Searching for mutations in the epigenetic machinery, two groups sequenced all exons of *MBD1*, *MBD2*, *MBD3*, and *MBD4*. The R269C mutation (*MBD1*) was reported in one autistic patient, although it was absent in the control group [Li et al., 2005]. The other group identified 46 alterations, although R269C was not among them. Variants in *MBD3* and *MBD4* were also described, suggesting that mutations in MBD genes might participate in the development of ASD [Cukier et al., 2010].

The new "omics" technologies have enhanced the race for the search and better understanding of the central nervous system as well as its pathologies. An exome-based sequencing study was carried out on 57 trios with schizophrenia and detected an increased proportion of de novo mutations. Additionally, there was an overlap between these mutated genes and the genes implicated in ASD, including *MeCP2* [McCarthy et al., 2014]. Another genomic study evaluating velo-cardio facial syndrome and increased risk for schizophrenia described, in a psychotic patient, a deletion at 2q23.1, which includes the *MBD5* locus and was previously linked to intellectual disability, epilepsy, and autistic features when deleted [Williams et al., 2013]. Recently, our group evaluated the effect of *MBD5* haploinsufficiency in neuronal progenitor cells, and reported decreased expression of genes implicated in cell division and proliferation and increased expression of genes involved in neural differentiation. Similar alterations were observed for miRNA expression pattern, therefore supporting the theory that regulation of proliferation and differentiation of neural stem cells underlies ASD, and epigenetic regulators, as MBD5, are fundamental to this pathway [Chen et al., 2014; Gigek et al., 2015].

Furthermore, GWAS was performed in children with social communication problems as reported by their parents. Among the top regions associated with this trait was 6p22.1, including *TRIM27*, which encodes an ubiquitin E3 ligase, a known interaction partner of MBD3 and MBD4 [St et al., 2013]. Taken together, these findings suggest that these psychiatric disorders share a genetic etiology [Gigek et al., 2015] and polymorphisms

and mutations in MBD genes might play a crucial role in the pathogenesis of these disorders.

More recently, variants in the epigenetic machinery were described and associated with their gene expression. Some of these variants led to abnormal gene expression and were claimed to be part of the etiology of these diseases. *MeCP2* mutations in the 3′UTR region were associated with lower expression levels in autistic patients [Coutinho et al., 2007].

Furthermore, *MBD4* expression was significantly upregulated in hippocampus [Benes et al., 2009] and prefrontal cortex of patients with schizophrenia when compared to controls, although *MBD4* levels did not differ between groups when another dataset was analyzed in the same study [Gavin et al., 2013].

Animal models were also used to evaluate the role of MBDs in psychiatric disorders. *Mbd1*-null mice exhibited several core deficits frequently associated with ASD, including reduced social interaction, anxiety and depression. It was reported that *Htr2c*, a serotonin receptor gene, is directly regulated by Mbd1 binding to the promoter region, and the loss of Mbd1 led to *Htr2c* overexpression [Allan et al., 2008]. Moreover, disrupted *MBD5* gene mice presented phenotypes similar to the observed in 2q23.1 deletion, thus demonstrating the relevance of this gene in the understanding of neurodevelopment [Camarena et al., 2014; Gigek et al., 2015].

Of equal importance are the targets of MBDs. Using MBD2 sequencing, it was possible to generate a methylome map in astrocyte-associated depression. In this case, the majority of the differentially methylated regions (DMR) displayed reduced methylation levels than those of controls. Among intragenic DMRs, *GRIK2* (glutamate receptor, ionotropic kainate 2) and *BEGAIN* (brain-enriched guanylate kinase-associated protein) were the most dramatic and showed significant correlations with gene expression [Nagy et al., 2014].

These findings suggest that perturbations in genes known to function in the epigenetic regulation of brain development and cognition could play a central role in the susceptibility to and pathogenesis and treatment of mental disorder [McCarthy et al., 2014].

FUTURE PERSPECTIVES

The MBDs are a class of key readers of DNA methylation that occurs in the gene and/or adjacent regions. In this review, we described the MBD family and the current knowledge on how they have an influence in cancer and psychiatric diseases. Literature data showed altered expression of a specific MBD itself can cause changes in the read-out of the DNA and trigger dysregulation of transcriptional activity. Furthermore, MBD variants have also been implied in some psychiatric diseases and cancer. These studies highlight the importance of MBDs in the epigenetic landscape. Comprehend which genes undergo regulation by specific MBD in the normal and pathological condition is the key to understand how this class of proteins cross-talk to epigenetic modifications and act in fine-tune gene expression to affect homeostasis in a specific cell or tissue type. In this sense, new and improved techniques, such as next generation sequencing and targeted expression silencing or overexpression can provide novel and better understanding of regions that undergo DNA methylation.

ACKNOWLEDGMENTS

C O Gigek has fellowship granted by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). M.A.C. Smith has research grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors thank their laboratory members for their encouragement and support.

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